

BRILLIANT GREEN AGAR MODIFIED

Powdered medium and ready to use plates of Edel and Kampelmacher selective medium for the isolation of *Salmonella* other than *S.typhi*

Typical formula (g/l)

Beef Extract	5.0000
Peptone	10.0000
Yeast Extract	3.0000
Disodium Hydrogen Phosphate	1.0000
Sodium Dihydrogen Phosphate	0.6000
Lactose	10.0000
Sucrose	10.0000
Phenol Red	0.0900
Brilliant Green	0.0047
Agar	13.0000

Directions for powdered medium

Suspend 52.7 g in 1000 ml of cold distilled water, heat to boiling with frequent agitation. Do not sterilise by autoclaving. Cool to 50°C and distribute into sterile Petri dishes.

Final pH 6.9 ± 0.1

Description

Brilliant Green Agar Modified is prepared according to the formulation of Edel and Kampelmacher. It is suitable for the detection of *Salmonella* spp. in food and animal feed stuffs. Brilliant green inhibits Gram-positive bacteria and most Gram-negative bacteria. Sucrose and lactose are present as fermentable carbohydrates. In comparison with Kristensen medium, this medium is enriched with nutritive factors and is buffered with phosphate salts.

Technique

For foodstuffs the following procedure is recommended:

In general, for the preparation of initial suspension add 25g of sample portion to 225ml of Buffered Peptone Water. If the required test portion is other than 25g, use the suitable quantity of Buffered Peptone Water to yield approximately 1/10 dilution (m/v).

Incubate the initial suspension at 37°C for not less than 16 hours and not more than 20 hours.

Transfer 0,1ml of the pre-enriched culture to a tube containing 10ml of Rappaport Vassiliadis (RV) Broth (cat. N° 401980) and 10ml to a flask containing 100ml of Selenite Cystine Broth (cat. N° 402026)

Incubate the inoculated RV Broth at 42°C for 24 h.

Incubate the inoculated Selenite Cystine Broth at 37°C for 24 h and a further 24hrs.

Using the culture obtained in the RV Broth inoculate by means of a 3mm loop a large-size Petri dish or two 90mm Petri dishes containing Brilliant Green Agar Modified.

Proceed in the same way from the tubes of RV Broth, inoculating a second plating medium (e.g. Chromogenic Salmonella Agar, cat. N° 405350), or other suitable selective *Salmonella* plating-out medium, has chosen by the laboratory.

Using the cultures obtained in the Selenite Cystine Broth after 24 and 48 hours of incubation, repeat the procedure with the same two selective plating-out media.

Invert the dishes and incubate at 37°C for 20-24 h. Examine for the presence of typical colonies.

On Brilliant Green Agar Modified, *Salmonella* grows with red colonies and the medium changes from pink to red.

Some strains of lactose and sucrose fermenting *Enterobacteriaceae*, grow with yellow colonies.

If growth is slight, or if no typical colonies are observed, re-incubate at 37°C for further 18-24hrs.

Any typical or suspected colony should be subjected to a biochemical and serological confirmation using a pure subculture in a nutrient agar plate.

Biochemical confirmation tests include: TSI Agar, Urea Agar, L-Lysine Decarboxylase Medium, detection of beta-galactosidase, VP reaction, indole detection.

Serological confirmation includes the detection of the presence of *Salmonella* O-, Vi and H antigens by slide agglutination test.

Biochemical confirmation could be substituted with the rapid test MUCAP (code 191500). All the colonies MUCAP positive must be serological confirmed.

User quality assurance (37°C-24 h)

Productivity control

S. enteritidis ATCC 13076: growth, red-purple colonies, surrounded by a diffused red halo*S. typhimurium* ATCC 14028: growth, red-purple colonies, surrounded by a diffused red halo

Selectivity control

S. aureus ATCC 25923: inhibited*E. faecalis* ATCC 29212: inhibited**Storage**

Dehydrated medium: 10-30°C

Ready to use plates: 2-8°C

User prepared plates: 1 month at 2-8°C

References. Edel, W. and Kampelmacher, E.H. (1968) Bull. WHO, **39** (3), 487.**Packaging****4012562****Brilliant Green Agar Modified,****500 g (9.5 l)****541256****Brilliant Green Agar,****20 ready to use plates**